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ROLE OF ANTITHROMBIN FOR INACTIVATION OF THROMBIN ON ENDOTHELIUM

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Inactivation of thrombin which occurs mainly on the endothelium requires binding of the enzyme to receptors on the endothelial cells. One receptor for thrombin is glycosaminoglycans (GAG). Antithrombin (AT), however, also binds to GAG and may be involved in endothelial inactivation of thrombin.

The purpose of the present study was to study the role of AT for the inhibition of thrombin on endothelium. The experiments were performed on rabbit aorta segment in vitro. Thrombin in solution and on the endothelial surface was functionally assayed with aid of a synthetic chromogenic substrate ($thrombin_S$) or fibrinogen ($thrombin_F$). In the latter case liberation of fibrinopeptide A (FPA) was measured. Inactivation of thrombin was estimated by the difference between loss of thrombin from the solution and recovery of thrombin on the endothelial surface during the incubation with thrombin.

Preincubation of the endothelium with AT or plasma increased the inactivation of both $thrombin_S$ and $thrombin_F$ but AT-free plasma had no such effect. Preincubation with heparin (5 IU ml) decreas- the inactivation of thrombin on the endothelium. Endothelial segments preincubated with AT caused a much more rapid inactivation of thrombin over time as compared to control segments. Aortic segments preincubated with heparin caused a slower inactivation of thrombin on the sruface as compared to control segments.

It is concluded, that preincubation with AT enhances the inactivation of thrombin on the endothelium whereas preincubation with heparin has the opposite effect. Heparin causes liberation of endogenous AT from the endothelial surface which may explain the decreased inactivation on the surface in the latter case.

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THE BINDING OF ANTITHROMBIN TO CAPILLARY ENDOTHELIAL CELLS GROWN IN VITRO.

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Cloned endothelial cells from rat epididymal fat pads synthesize anticoagulantly active heparan sulfate proteoglycans containing the disaccharide, GlcA→AMN-3,6-0-S03, which is a marker for the antithrombin-binding domain of heparin. To demonstrate that antithrombin (AT) binds to cell surface heparan sulfate, a binding assay employing ^{125}I-AT and cell monolayers has been developed. Post-confluent endothelial cells (7 days) were incubated with radiolabeled AT for 1 h at 4° and washed with PBS. Bound radioactivity was quantitated after solubilizing whole cells. Under these conditions, ~1% (2174±50 cpm/5x10^4 cells) of the ^{125}I-AT bound to the endothelial cell monolayer, whereas none of the radiolabeled protein bound to CHO cells or bovine smooth muscle cells. Utilization of unlabeled AT (1 µM) in experiments conducted as described above resulted in a reduction (73%) of the binding of the labeled species to endothelial cells. To assess whether heparan sulfate was responsible for AT binding, cell monolayers were incubated for 1 h at 37° with purified *Flavobacterium* heparinase (0.2 units). Over 90% of ^{125}I-AT binding to these cellular elements was suppressed with the bacterial enzyme. Internalization of radiolabeled AT by endothelial cells was examined by incubating the protease inhibitor and cells at 4° and 37°. An initial rapid binding was observed at both temperatures. At 4° AT binding plateaued within 15 min, whereas at 37° binding did not plateau until 60 min and was 30% greater than that observed at 4°. These data suggest that surface-associated AT can be internalized by endothelial cells. In addition, AT binding was shown to increase with the length of endothelial cell post-confluence, indicating an accumulation of heparan sulfate by these cells during quiescence. In conclusion, our studies support the hypothesis that the vascular endothelium is coated with heparan sulfate-bound AT, which is responsible for the antithrombotic properties of these natural surfaces.

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ALTERED INTERACTION OF THROMBIN AND ANTITHROMBIN III WITH THE VASCULAR WALL.

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The role of the endothelial injury in the development of the thrombophylic state was studied in rats with nephrotic syndrome (NS,Heymann nephritis).There were a 6-fold increase of the soluble fibrin concentration and a 30% decrease of plasma antithrombin III (AT) activity in the NS.It was found that the plasma half-life of ^{125}J-labelled α-thrombin (10^{-7} M) is 3,0 ± 0,6 min in control animals and 4,0 ± 0,1 min in NS rats.At 20 min following the administration of bovine ^{125}J-thrombin it was observed that in normal animals 84% of the radiolabelled enzyme was bound with vessel wall,while in NS rats the figure was only 65% (p< 0,05).The alteration of thrombin binding to the vascular wall lead to an increase in the amount of soluble fibrin-monomer and AT-proteinase complexes.AT-thrombin complexes and a proteolytically modified form of AT (Mr<68 kDa) were isolated from NS rats plasma by affinity chromatography on heparin-sepharose and chromatofocusing.At 5 min following injection of a 100-fold molar excess of bovine AT (1,7 . 10^{-5} M) it was observed that 35% of thrombin reversibly bound to the endothelium could be detected in the circulation of normal rats.The same excess of AT induced only a 10% (p<0,001) release of ^{125}J-thrombin to the blood stream in the NS rats through the formation of ^{125}J-thrombin complexes with Mr≥100 kDa.It is being proposed that injury of the vascular wall in the NS animals facilitated the interaction of the enzyme with the substrate (fibrinogen) and inhibitor (AT), and leads to ineffective inactivation of thrombin bound to the endothelium by AT.

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THE SIGNIFICANCE OF VON WILLEBRAND FACTOR ANTIGEN (vWFAg) AS AN ENDOTHELIAL MARKER DURING HAEMODIALYSIS.

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It has been suggested that platelet activation during haemodialysis (HD) is associated with damage to the endothelium which might contribute to increased atheroma seen in HD patients. We have therefore measured vWFAg (by radial immunodiffusion) as an endothelial marker during HD and B-thromboglobulin (BTG) (by RIA) as a marker of platelet activation with various anticoagulant regimes: unfractionated heparin (UFH) (Leo), low molecular weight heparin (LMWH) (Choay CY 216), regional citrate anticoagulation, prostacyclin (PGI2) (5ng/kg/min) and the combination of PGI2 + UFH.

Platelet activation occurred (as shown by a progressive rise of BTG) during HD with UFH, LMWH and citrate, but not with PGI2 or PGI2 + UFH. Pre-dialysis vWFAg levels were elevated in HD patients (2.37 ± 1.16 (SD) U/ml) compared with healthy controls (1.15 ± 0.34 U/ml, p<0.001). Plasma concentrations of vWFAg increased during HD with UFH (26 ± 13% rise after 120 mins, p<0.001), but not during HD with LMWH, citrate, PGI2 or PGI2 + UFH. UFH infusion without HD did not result in vWFAg rise nor did UFH or LMWH interfere in vitro with the vWFAg assay. Thus PGI2 prevented both platelet activation and release of vWFAg. With LMWH and citrate there was no increase of vWFAg despite evidence of platelet activation.

Elevated pre - dialysis vWFAg concentrations in HD patients may reflect chronic endothelial injury or alternatively may be due to impaired clearance of vWFAg in uraemia. The results obtained during HD suggest that vWFAg release occurred only in the presence of both platelet activation and high molecular weight heparin chains. Whether the release of vWFAg reflects disruption and damage to the endothelium or stimulation and displacement of vWFAg remains uncertain.

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